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Conformational Characterization of Tachykinin Neuropeptides: Role of the Polyproline II Structure
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Substance P (SP), Neurokinin A (NKA) and Scyliorhinin I (Scyl) peptides belong to the Tachykinin family and are agonists for Neurokinin1 (NK1) GPCR receptor with different potent activities. These Tachykinins are involved in several physiological processes and some neurodegenerative disorders, what make them relevant therapeutically agents. The characterization of the active peptide conformations of the Tachykinins is essential for the elucidation of the mechanism of action and for the design of drugs. To address the molecular basis for the peptide recognition by the NK1 receptor we studied the conformation of SP, NKA and Scyl in solution and membrane-mimic environments: micelles and liposomes, by using CD and FTIR spectroscopies. The analysis of CD data revealed that the three peptides form a partially α helical structure in the presence of the negatively charged micelles and vesicles, but not in zwitterionic DMPC. By performing CD spectra at increasing temperatures we demonstrated that in an aqueous environment SP, NKA and Scyl form extended polyproline II (PPII) helical structure. The same structure was found in the membrane mimics, which do not induce formation of α helical conformation of the peptides. FTIR experiments performed in D_2O support the presence of PPII conformation. Further we questioned whether the formation of PPII needs peptide binding to the membrane or it is a peptide intrinsic property, by using fluorescence techniques. We propose that the peptide-membrane interaction is a two-step process involving first electrostatic interactions through PPII structure, followed by the folding and insertion of the alpha-helical segment.

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Bicelle-Bound Solid-State NMR Structural Studies and Membrane-Permeabilizing Activities of Piscidin 1 and Piscidin 3: Implications for Mode of Antimicrobial Action
Matthew K. Baxter, Jason A. McGavin, Nina B. Kraus, Anna A. De Angelis, Jolita Seckute, Caitlin Burzynski, Daryl M. Berke, Nedzada Smajic, Linda K. Nicholson, Stanley J. Opella, Myriam Cotten.
Bicelles represent a novel preparation of hydrated lipid bilayers, which can be used to study membrane-associated proteins under physiologically-relevant conditions. Large bicelles can be oriented within a magnetic field, enabling the determination of high-resolution peptide structures and angles of insertion within a lipid membrane via solid-state Nuclear Magnetic Resonance (NMR). Piscidin, an amphipathic, antimicrobial peptide found in hybrid striped bass, plays a major role in host defense. It is effective against a wide range of pathogens, including methicillin-resistant Staphylococcus aureus and HIV-1. The peptide is known to have an alpha-helical conformation when bound to anionic lipid membranes that mimic the surface of bacterial membranes.
We have investigated the use of bicelles in the study of piscidin. 15N NMR spectra show that piscidin has been successfully aligned in magnetically oriented bicelles. 31P NMR studies, which show that piscidin disrupts bicelle-forming lipids, have helped us better understand its mode of action. To complement these backbone solid-state NMR studies of piscidin, we have used fluorescent-dye leakage experiments with various phospholipids and have performed solution NMR to determine the charge state of the histidine side chains in the presence of micelles and investigate their possible role in mediating important peptide-lipid interactions. The long term goal of this project is to improve our understanding of structure function relationships in an interesting family of antimicrobial peptides. This knowledge could be used to design potent antimicrobial pharmaceuticals that minimize bacterial resistance.

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Atomic-Resolution Three Dimensional Structures and Membrane Locations of Antimicrobial Piscidin 1 and Piscidin 3 in Aligned Lipid Bilayers: A Solid-State NMR and Molecular Dynamics Investigation
Myriam Cotten, William E. Wieczorek, Mukesh Sharma, Milton Truong, Breanna S. Vollmar, Eric D. Gordon, Richard M. Venable, Richard W. Pastor, Riqiang Fu.
Piscidin, an amphipathic cationic antimicrobial peptide (AMP) active against a broad range of pathogens including multidrug-resistant bacteria and HIV-1, belongs to a large family of vital host-defense peptides that interact, at least initially, with negatively-charged microbial membranes in order to perform their function. While two piscidin isoforms, piscidin 1 (p1) and piscidin 3 (p3), are highly homologous, they display unequal antimicrobial and hemolytic effects. As a way to identify factors optimizing specific molecular interactions directly related to their mode of membrane activity, we have investigated p1 and p3 bound to lipid membranes that mimic bacterial membranes.
Previously, we used solid-state NMR on 15N-labeled peptides to demonstrate that membrane-bound p1 and p3 adopt an alpha-helical structure and lie in the plane of hydrated lipid bilayers where they experience fast dynamics. Our recent analysis of two-dimensional solid-state NMR data has lead to the first atomic resolution three-dimensional backbone structures of p1 and p3 bound to aligned lipid bilayers. Structural calculations based on the NMR data and molecular dynamics simulations have been performed to yield a refined structure and membrane location for each peptide.
We will explain how our atomic-level investigation of the structure, dynamics, and bilayer location of piscidin provides new insights into its mode of action and therefore allows us better to understand how AMPs disrupt bacterial membranes and induce cell death. The long term goal is to derive common principles that could facilitate the design of pharmaceuticals with enhanced antibacterial activity and lower toxicity on mammalian cells.

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The Importance of the Proline Hinge in the Action of Histone-Derived Antimicrobial Peptides
Kathryn E. Pavia, Sara A. Spinella, Kathy J. Chen, Donald E. Elmore.
Antimicrobial peptides (AMPs) are short, polycationic proteins capable of killing a wide variety of bacterial species through a number of different mechanisms. The ability to effectively engineer potent, cell-penetrating AMPs would maximize the full potential of these molecules. Buforin II (BF2), a cell-penetrating histone-derived antimicrobial peptide (HDAP), served as a model for the design of three novel histone-derived peptides, DesHDAPs1-3. BF2 is has a C-terminal α-helix that is broken by a proline hinge. Because this proline hinge determines, in part, BF2's ability to translocate into and kill bacterial cells, DesHDAPs1-3 were designed to also contain a helix-breaking proline residue. To determine whether this structural feature plays the same role in the designed peptides, the activity of proline to alanine mutants of each designed peptide were tested against a variety of bacterial species. As expected, circular dichroism measurements indicate that the proline to alanine mutation increases the α-helical character of BF2 and all designed peptides. For both BF2 and DesHDAP1, proline to alanine mutants show decreased antimicrobial activity against all species tested. In contrast, proline to alanine mutations in both DesHDAP2 and DesHDAP3 either do not affect or slightly increase the observed antimicrobial activity. This suggests that α-helicity is a poor predictor of antimicrobial activity for this family of HDAPs, and that the proline residue may play a different role in DesHDAP2 and DesHDAP3 than it does in BF2 and DesHDAP1. In order to explain these trends, we have further characterized the translocation and membrane permeabilization properties of the proline to alanine mutations. As well, we have used molecular dynamics simulations to explore the structure of the membrane bound peptides.

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Structure of Peptide-Induced Transmembrane Pore Determined by Anomalous X-Ray Diffraction
Ming-Tao Lee, Shiuan-Shiaou Wu, Wei-Yu Lin, Yi-Ting Sun, Wei-Chin Hung.
We determined the structure of the melittin-induced transmembrane pore by X-ray diffraction. The multibilayer sample on substrate was prepared in full hydration. The peptide-to-lipid ratio, P/L, of the melittin-lipid mixtures were in the condition where pores were present, as established previously by neutron in-plane scattering in correlation with oriented circular dichroism. At low hydration levels, the interbilayer distance shortened and caused the membrane pores to become long-ranged correlated and form a periodically ordered lattice of rhombohedral symmetry. Here we used the multiwavelength anomalous dispersion (MAD) method to solve the phase problem for a rhombohedral phase of a phospholipid with brominated chains and performed multiwavelength anomalous diffraction at the bromine K edge. The X-ray light source in BL23A beam line of NSRRC and home-made humidity-temperature controlled chamber will be applied in the measurements. We found the melittin-induced pores were at least partially framed by a lipid monolayer. Evidence suggests that the pore structure is of the toroid type different from the barrel-stave type induced by alamethicin.

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High Throughput Screen of Combinatorial Peptide Library for Gain-of-Function and Loss-of-Function Changes to Melittin
Aram J. Krauson, William C. Wimley.
Melittin, the main peptide component of European Honey Bee venom, is an amphipathic, 26-amino acid peptide that lyses bacterial and mammalian cells by forming transmembrane pores. Our research focuses on the various mechanisms of peptide permeation of membranes. We have designed orthogonal, fluorescence-based assays to characterize long-lived pore-forming peptides such as melittin. In these assays, peptides are incubated overnight with vesicles containing dye-labeled lipids and entrapped terbium. In the first measurement, the sum of the lytic activity is determined by measuring the terbium released from the vesicles. In the second measurement, we add